Modularly Constructed RNA Molecules Having two Sequence Region Types

The present invention relates to RNA molecules which are characterized by two sequence region types, namely a first sequence region type which contributes to maintaining the three-dimensional structure of the RNA molecule, and a second sequence region type which is responsible for the specific binding of a ligand. These RNA molecules are preferably useful for the direct control of gene expression. The present invention also provides the DNA sequence derived for the RNA molecules according to the invention and vectors which contain them. In addition, the invention relates to drugs or medicaments and diagnostic compositions which contain the above RNA molecules or vectors, to an antibody specifically recognizing these RNA molecules or to antisense RNA specifically binding to these RNA molecules or ribozymes cleaving these RNA molecules. Furthermore, the invention relates to non-human transgenic mammals and cells obtained therefrom.

Gene expression in eukaryotes is usually regulated via proteins which usually bind specifically to certain regulatory sequences upstream of the gene to be expressed and show a characteristic effect (RNA polymerases, transcription factors, receptors adapted to be activated by hormones, etc.). Only few examples of controlling the gene expression directly via RNA molecules have been known thus far. They include the RNA "XIST" responsible for the inactivation of the entire X chromosome ("X chromosome inactivation specific transcript"), an RNA referred to as

IPW ("imprinted in Prader-Willi syndrome") and RNA H19 which represents a tumor suppressor and is involved in the control of certain development processes. The artificial control of the gene expression has meanwhile been effected by the use of antisense RNAs binding specifically to mRNAs or by the use of catalytically active RNA molecules, what is called ribozymes, which do not only bind specifically to the target RNA but also cleave it thus inactivating it. However, the application possibilities for these antisense RNAs or ribozymes are limited, above all as regards the ligand to be bound and inactivated. This ligand may basically only be an RNA.

Thus, there is a need for providing compounds which can universally detect, and/or inactivate, the most differing target molecules, e.g. DNA, RNA, proteins or low-molecular substances, and are suitable e.g. for controlling gene expression and thus, of course, also for preventing and treating diseases which are accompanied by a disturbed gene expression.

Hence the technical problem of the invention is substantially to provide those compounds which are useful *inter alia* for the prevention or therapy (and also diagnosis) of such diseases.

The solution to this technical problem was achieved by providing the embodiments characterized in the claims.

The inventors could identify an RNA molecule which comprises the above described desired properties. This RNA molecule is encoded by the gene "NINTROX" (No INTROns X-chromosome) which has no introns, is localized on the X-chromosome and codes for no protein. This RNA molecule is part of certain

(relatively long) transcripts of the MeCP2 gene. The MeCp2 gene (methyl-CpG binding protein 2) in Xq28 has a transcript of about 1.8 kb which codes for the MeCP2 protein. The above described RNA is part of relatively long MeCP2 transcripts which also code for the MeCP2 protein but have a different 3'-non-translated region. This 3'-non-translated region is decisive for the MeCP2 gene and its function. The below expression "NINTROX" is synonymous with the above relatively long transcripts of the MeCP2 gene.

The genomic sequence of the human NINTROX gene is shown in figure 1, and the genomic sequence of the murine NINTROX gene is illustrated in figure 2. In figure 3, a sequence comparison was carried out between human and murine sequences. It is obvious therefrom that there are some highly sequence-conserved regions which according to an energy analysis carried out by means of a computer distinguish themselves by a high degree of energy (cf. figure 4).

While the mechanism of action of the above discussed genes effective on the RNA level was fully unclear, the principle of action of such a gene which is described in more detail below could, for the first time, be determined by the analysis of the NINTROX gene. The NINTROX gene contributes essentially to the maintenance of the functions of the CNS, in particular the hippocampus. Defects in this gene result in limited CNS functions which reach as far as mental retardations. Furthermore, the NINTROX gene has an important function in the control of cell proliferation. In this connection, changes in this gene can lead to errors in the control of cell growth, e.g. to cancer. Changes in this gene may result in an increased or reduced DNA methylation. An increased DNA methylation can *inter alia* restrict or prevent

the activity of growth-controlling genes (tumor suppressor genes) and thus result in a generally increased cancer rate. Reduced DNA methylation can lead *inter alia* to an overexpression of genes and thus to a disturbed development of the cell or the whole organism. Further investigations led to the result that the expression pattern of the NINTROX gene is effected in tissue-specific and development-specific manner. The Northern analyses showed an expression in all investigated fetal and adult tissues. No sequence homologies with already known sequences could be detected.

The strategy which led to the identification of this nucleic acid molecule is described below. Based on the systematic analysis of the q28 region of the human X chromosome various expressed sequences could be detected and isolated. By means of these expressed sequences some formerly unknown genes could be identified and characterized according to standard methods, *inter alia* the NINTROX gene on which the present invention is based.

It is of interest that the NINTROX-RNA molecules according to the invention have a modular structure, i.e. they are characterized by the presence of two different sequence region types. While one sequence region permits to maintain the three-dimensional structure and, as follows from a comparison of the sequences from various species (human, hamster, kangaroo, macaque or macaca, orangutan chimpanzee and rat; cf. figure 5), is conserved only in a qualified sense, the second sequence region which is responsible for the specific binding to the target molecule is sequence-conserved. Because of this modular construction of the NINTROX-RNA it is possible to modify it such that its effect is not only limited to the above described control of the gene expression but can be used for a number of

possibilities. In addition to the control of the gene expression it is also possible to modify the structure (e.g. chromatin structure, nuclear scaffold) of chromosomal regions by means of such modular RNA molecules. This offers the formerly unknown possibility of being able to influence the expression of relatively large genomic regions in well-calculated fashion. Thus, certain sequence regions of both modules of the NINTROX gene can be replaced by other sequences or even artificial sequences, so that (a) the interaction of this RNA with other binding partners (RNA, DNA, other macromolecules and low-molecular compounds) or their biochemical reaction (e.g. increase or decrease of the conversion rate) are changed in well-calculated fashion, and therefore the RNA molecule can be adapted in well-calculated fashion to novel tasks, and/or (b) the three-dimensional structure of the NINTROX-RNA can be adapted in well-calculated fashion to special demands. As a result, a partially or fully new function of the NINTROX-RNA molecule according to the invention can be obtained.

Thus, an embodiment of the present invention relates to an RNA molecule which may bind to a ligand and comprises the following sequence regions: (a) a sequence region maintaining the three-dimensional structure of the RNA molecule, and (b) a sequence region for the specific binding of the ligand.

The expression "a sequence region maintaining the three-dimensional structure of the RNA molecule" used herein has the following meaning. Three-dimensional RNA structures are rendered possible by base pairing of various bases within the RNA molecule. In this case, structures such as "stems" or "loops" are formed. Many of these structures yield in this way the overall structure of the RNA molecule. A

sequence change within the RNA molecule may remain without consequences for the spatial structure if the sequence change does not change the base pairings or if the sequence change is compensated by a second sequence change. For example, if the base pairing A-T is destroyed in that the A mutates into G, this mutation can be compensated by another mutation of T into C. Although this changes the sequence, the spatial structure remains the same. As a result, the same RNA structure can be formed by an extremely large number of differing RNA sequences. References to certain RNA structures follow from an analysis of the energy included therein. This analysis can be carried out by means of commercially available computer programs (e.g. "FOLD"; Michael Zuker and P. Stiegler: Optimal Computer Folding of Large RNA Sequences Using Thermodynamics and Auxiliary Information, Nucleic Acids Research (81), 9(1), page 133). The lower the energy content of a certain sequence, the more stable the three-dimensional RNA structures. The analysis of the NINTROX gene showed a conserved distribution of these low-energy structures (figure 4). The base sequence of these RNA regions differs widely with various species, but the energy content is very conserved. In figure 3, these are the sequence regions which are not characterized by a black bar at the margin. This means that the sequence region maintaining the three-dimensional structure of the RNA molecule is not sequence-conserved but energy-conserved. For example, modifications of this sequence region do not orient themselves by the base sequence but by the conservation of the detected energy content.

The expression "a sequence region for the specific binding of the ligand" used herein relates to a sequence region which is such that it can bind specifically the desired ligand. These sequence regions are highly sequence-

conserved. In figure 3, these regions are marked by a black bar at the margin and have a high energy content (cf. figure 4). This tallies with the observation that these sequence regions are not "packed" but oriented outwardly and are responsible for the binding of the ligand, enzymatic reactions or the binding to other RNA or DNA sequences. If the ligand to be bound is an RNA molecule or a DNA molecule, this sequence region will be complementary to a corresponding, sufficiently long segment of the RNA molecule or DNA molecule. If the ligand to be bound is a protein, the sequence region (b) may be partially or fully exchanged, or supplemented, by a DNA sequence which as is known binds specifically the desired protein.

The two above-described sequence types occur several times within the NINTROX-RNA. The exchange or the change of individual ones of such modules enables the well-calculated change of the NINTROX-RNA. In a modification of the module maintaining the three-dimensional structure attention has to be paid to the energy content, so that it maintains a minimum value. The modification of the other sequence region is only subject to minor restrictions even though it is deemed to be sequence-conserved. This region may be omitted fully or partially or may contain insertions. For example, it is also possible to insert sequences into the NINTROX-RNA molecule which have known biochemical properties or bind certain DNA molecules, RNA molecules or proteins. In addition, random sequences of differing length may be introduced into various sites of the NINTROX gene and thereafter selection for specific properties such as biochemical reaction, specific binding, etc., may be carried out.

In a preferred embodiment of the RNA molecule according to the invention the sequence region (a) comprises the sequence regions not marked at the margin in figure 3 or sequences related thereto which also permit the maintenance of the three-dimensional structure of the RNA molecule and differ from sequence region (a) in figure 3. These differences relate to the addition, deletion and/or insertion of bases, at least 80 %, preferably 85 %, and more preferably at least 90 %, of the energy content determined for the sequence of figure (3) being maintained. The original three-dimensional structure is preferably maintained when these changes are introduced.

In a particularly preferred embodiment, the sequence region (b) of the RNA molecule according to the invention comprises the sequences which are illustrated in figure 3 and marked with black bars at the margin.

In another preferred embodiment of the RNA molecule according to the invention, the ligand to be bound is a DNA molecule or a protein or enzyme, e.g. DNA polymerase I. The RNA molecule according to the invention preferably contains a poly(A) sequence at the 3' end, which may contribute to the stability in a desired host cell.

In another preferred embodiment, the RNA molecule according to the invention is used to control the gene expression. For this purpose, the sequence region (b) is modified such that it binds a protein responsible for gene expression or binds to a certain DNA region of the target gene so as to impede or prevent e.g. the attachment of proteins which exert an influence inhibiting or supporting gene expression or also binds directly to the mRNA of the target gene so as to impede or prevent the translation, for example. The person

skilled in the art can readily modify the RNA molecule according to the invention by corresponding modification of sequence region (b) and possibly also of sequence region (a) such that it binds the desired ligand and therefore controls the gene expression to the desired extent.

The present invention also relates to a DNA sequence coding for the RNA molecule according to the invention and to a gene comprising the following features: It contains a promoter which permits the transcription in a desired host cell and a DNA sequence functionally linked therewith and encoding the RNA molecule according to the invention. The gene preferably contains additionally a termination signal and a polyadenylation site.

In a preferred embodiment, the gene according to the invention comprises the sequence shown in figure 1 or 2.

The DNA sequences or genes, coding for the RNA molecule according to the invention, may also be inserted in a vector. Thus, the present invention also comprises vectors containing these DNA sequences or genes. The term "vector" relates to a plasmid (e.g. pUC18, pBR322, pBlueScript), to a virus or another suitable vehicle. In a preferred embodiment, the sequence coding for the DNA molecule according to the invention is functionally linked in the vector with regulatory elements which permit its expression in prokaryotic or eukaryotic host cells. In addition to the regulatory elements, e.g. a promoter, such vectors typically contain a replication origin and specific genes which permit the phenotypic selection of a transformed host cell. The regulatory elements for the expression in prokaryotes, e.g. *E. coli*, comprise the lac, trp promoter or T7 promoter, and those for the expression in eukaryotes comprise the AOX1 or

GAL1 promoter in yeast and those for the expression in animal cells comprise the CMV, SV40, RVS-40 promoter, CMV or SV40 enhancer. Further examples of suitable promoters are the metallothionein I and the polyhedrin promoters. Suitable vectors are e.g. expression vectors, based on T7, for the expression in bacteria (Rosenberg et al., Gene 56 (1987), 125), pMSXND for the expression in mammalian cells (Lee and Nathans, J. Biol. Chem. 263 (1988), 3521) and vectors derived from baculovirus for the expression in insect cells.

In a preferred embodiment, the vector containing the sequences coding for the RNA molecules according to the invention is a viral vector, e.g. a vaccinia virus or adenovirus, which is of use for a gene therapy. RNA viruses, above all retroviruses, are particularly preferred. Examples of suitable retroviruses are MoMuLV, HaMuSV, MuMTV, RSV or GaLV. For the purpose of gene therapy the RNA molecules according to the invention can be transported to the target cells in the form of colloidal dispersions as well. They comprise e.g. liposomes of lipoplexes (Mannino et al., Biotechniques 6 (1988), 682).

General methods known in the art can be used for constructing expression vectors which contain the sequences coding for the RNA molecules according to the invention and suitable control sequences. These methods comprise e.g. *in vitro* recombination techniques, synthetic methods and *in vivo* recombination methods, as described in Sambrook et al., for example.

The present invention also relates to host cells containing the above described vectors. These host cells comprise bacteria, yeast, insect and animal cells, preferably mammalian cells. Preferred mammalian cells are CHO, VERO,

BHK, HeLa, COS, MDCK, 293 and WI38 cells. Methods of transforming these host cells, of phenotypically selecting transformants and expressing the nucleic acid molecules according to the invention using the above described vectors are known in the art.

The present invention also relates to antibodies which detect specifically the RNA molecule according to the invention. The antibodies may be monoclonal, polyclonal or synthetic antibodies or fragments thereof, e.g. Fab, Fv or scFv fragments. In this case, a monoclonal antibody is preferably concerned. The antibodies according to the invention may be produced according to standard methods, the RNA molecule according to the invention or a fragment thereof serving as an immunogen. Monoclonal antibodies may be produced e.g. by the method described by Köhler and Milstein (Nature 256 (1975), 495) and Galfré (Meth. Enzymol. 73 (1981), 3), mouse myeloma cells being fused with immunized mammalian spleen cells. These antibodies may be used e.g. to inhibit the activity of the RNA molecules according to the invention, e.g. to influence the gene expression. The antibodies may also be used in diagnostic assays, for example, so as to prove whether dysregulation of the gene expression is accompanied e.g. by a loss or lack of responsible NINTROX-RNA. The antibodies may be present in immunoassays in liquid phase or be bound to a solid carrier. In this connection, the antibodies may be labeled in various ways. Suitable markers and labeling methods are known in the art. Examples of immunoassays are ELISA and RIA.

The invention also relates to antisense RNAs which bind specifically to an RNA molecule according to the invention and may be used *in vitro* or *in vivo* to reduce the expression of genes controlled directly by RNA, e.g. NINTROX-RNA. The

administration of the antisense RNA according to the invention to a target cell results in a reduced gene expression and is particularly useful for treating diseases which are characterized by an excessively great gene expression of the directly RNA-controlled gene (e.g. cancer diseases). In this connection, the antisense RNAs can be administered directly or as a DNA encoding the same, preferably inserted in a suitable vector. The suitable vectors comprise all of the vectors described above already in connection with the RNA molecules according to the invention.

The antisense RNAs according to the invention comprise an antisense sequence having at least 7 to 10 or more nucleotides which hybridize specifically with a sequence of the RNA molecule according to the invention, e.g. NINTROX-RNA. The antisense RNA according to the invention preferably has a length of about 10 to about 50 nucleotides or of about 14 to about 35 nucleotides. In further embodiments, the antisense RNAs according to the invention are RNAs shorter than about 100 nucleotides or shorter than about 200 nucleotides. In general, the antisense RNAs should be long enough to form a stable double helix but short enough (depending on the kind of supply) to be administered *in vivo*, if desired. In general, the antisense sequence is substantially complementary to the target sequence to ensure specific hybridization. In certain embodiments the antisense sequence is directly complementary to the target sequence. However, the antisense RNAs may also contain nucleotide substitutions, additions, deletions, transitions, transpositions or modifications as long as the specific bond to the relevant target sequence is maintained as a functional property of the antisense RNA. The antisense RNAs may also contain further sequences in addition to the

antisense sequences. The antisense RNAs (and the RNA molecules according to the invention) can be produced using any method suitable for the production of nucleic acids, e.g. by chemical synthesis *de novo* or by cloning. An antisense RNA may also be produced e.g. by inserting in a vector (e.g. a plasmid) a sequence of the target RNA or a fragment thereof in reverse orientation functionally linked with a promoter. Provided that the promoter and preferably termination and polyadenylation signals are positioned correctly, the strand of the inserted sequence is transcribed which corresponds to the non-coding strand acting as an antisense RNA.

The present invention also relates to ribozymes which cleave specifically the RNA molecules according to the invention and thus are also of use for inhibiting the gene expression. Useful ribozymes may comprise 5'-terminal and 3'-terminal sequences which are complementary to the target RNA, and they can be constructed by a person skilled in the art according to standard methods (see e.g. PCT publication WO 83/23572). The ribozymes according to the invention comprise e.g. ribozymes having the features of group I intron ribozymes (Cech, biotechnology 13 (1995), 323) and "hammerhead" ribozymes (Edgington, Biotechnology 10 (1992), 256).

In one embodiment, the ribozymes according to the invention *per se* are used as drugs. In another embodiment, gene therapy methods are employed for the expression of ribozymes in a target cell *ex vivo* or *in vivo*. The methods of administering the ribozymes or of expressing the ribozymes *in vivo* correspond to the methods described above in connection with the RNA molecules according to the invention.

The isolation and characterization of the human NINTROX gene and in particular the mouse homolog of the NINTROX gene allow to establish an animal model which permits to provide therapies and drugs for the above discussed diseases. Providing the sequence of the NINTROX gene enables both diagnosis (post-natally or pre-natally) and therapy of diseases in which the gene expression is characterized by the lack of NINTROX-RNA or an excess of NINTROX-RNA. However, the therapeutic or diagnostic application is not only limited to diseases, which are accompanied by a dysregulation of the expression of a gene controlled by NINTROX-RNA but the RNA molecules modified in accordance with the above described possibilities also offer the chance of using completely new therapeutic agents.

Therefore, the present invention also relates to drugs which contain the above described RNA molecules, vectors, antibodies, antisense RNAs or ribozymes. These drugs optionally contain additionally a pharmaceutically acceptable carrier. The person skilled in the art is familiar with suitable carriers and the formulation of such drugs. Suitable carriers include e.g. phosphate-buffered common salt solutions, water, emulsions, e.g. oil-in-water emulsions, wetting agents, sterile solutions, etc. The drugs can be administered orally or parenterally. The topical intra-arterial (e.g. directly to the tumor), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal or intranasal administration belong to the methods for the parenteral administration. A suitable dose is determined by the attending physician and depends on various factors, e.g. on the age, sex, patient's weight, stage of a tumor, kind of administration, etc.

The drug according to the invention is used preferably for preventing or treating diseases which are correlated with a disturbed control of gene expression. The drug according to the invention is used particularly preferably for treating tumoral diseases or diseases of the CNS. In this connection, the drug may be used in gene therapy, the above described methods or vectors being usable for introducing the nucleic acids according to the invention. On the other hand, the RNA molecule according to the invention may be administered directly so as to restore normal expression of the gene in cells which no longer have functional copies of the RNA molecule.

The present invention also relates to a diagnostic composition which contains the RNA molecule according to the invention, to the DNA sequence coding for it or a fragment thereof, to the antibody according to the invention or a fragment thereof, or to the antisense RNA according to the invention or a fragment thereof, or to combinations thereof, optionally together with a suitable analytical reagent. By means of this diagnostic composition the detection may be made as to whether the RNA directly controlling the gene expression, e.g. NINTROX-RNA, is present or, as compared to a control, is available in excessively high or low concentration or with a deviating length. In this connection, the antibody or a fragment thereof is preferably used in the above described assays or the antisense RNA or a fragment thereof as a probe in hybridization experiments. For this purpose, the probe preferably has a length of at least 10, more preferably at least 15, bases. Suitable detection methods based on hybridization are known to the person skilled in the art. Suitable labeling for the probe are also known to the person skilled in the art and they comprise e.g. labeling using radioisotopes, bioluminescence,

chemiluminescence, fluorescence markers, metal chelates, enzymes, etc. This process may use methods known to the person skilled in the art as regards the preparation of whole RNA or poly(A)+RNA from biological samples, the separation of the RNAs on gels separating according to size, e.g. denaturing agarose gels, the production and labeling of the probe and the detection of the hybrids, e.g. via "Northern blot". In this connection, diseases are preferably diagnosed as described above in connection with the drugs according to the invention.

A diagnosis can also be made on a DNA level. In this connection, the intactness of the gene which codes for the RNA which is directly involved in the regulation of gene expression, e.g. NINTROX-RNA, is investigated by the above described nucleic acid molecules (e.g. as regards the availability, length or mutations). For this process it is possible to use methods with which the person skilled in the art is familiar as to the preparation of DNA from biological samples, the restriction digestion of the DNA, the separation of the restriction fragments on gels separating according to size, e.g. agarose gels, the production and labeling of the probe and the detection of hybridization, e.g. via "Southern blot". The above detection can also be carried out via PCR. In this connection, primers are used which flank the coding sequence. Here, amplification products of DNA from the tissue in question, which differ e.g. as regards the length or sequence from the amplification products of DNA from healthy tissue, are of diagnostic significance.

The subject matter of the present invention also relates to a non-human mammal whose NINTROX gene is modified, e.g. by

insertion of a heterologous sequence, in particular a selection marker sequence.

The expression "non-human mammal" comprises any mammal whose NINTROX gene may be modified. Examples of such mammals are mouse, rat, rabbit, horse, cow, sheep, goat, monkey, pig, dog and cat, with mouse being preferred.

The expression "NINTROX gene which is modified" signifies that in the NINTROX gene naturally occurring in a human mammal a deletion of about 1 to 2 kb is carried out by standard methods. If desired, a heterologous sequence, e.g. a construct for mediating antibiotic resistance (e.g. a "neo cassette") can be inserted in this deletion. This method is generally described in Schwartzberg et al., Proc. Natl. Acad. Sci. USA, Vol. 87, pp. 3210-3214, 1990, to which reference is made.

A further subject matter of the present invention relates to cells which are obtained from the above non-human mammal. These cells may be present in any form, e.g. in a primary or long-term culture.

A non-human mammal according to the invention can be provided by common methods. A method is favorable which comprises the steps of:

(a) preparation of a DNA fragment, in particular a vector, containing a modified NINTROX gene, the NINTROX gene having been modified by deletion of a homologous sequence and/or insertion of a heterologous sequence, in particular a selectable marker;

(b) preparation of embryonal stem cells from a non-human mammal (preferably mouse);

(c) transformation of the embryonal stem cells of step (b) with the DNA fragment from step (a), the NINTROX gene in the embryonal stem cells being modified by homologous recombination with the DNA fragment from (a);

(d) culturing the cells from step (c);

(e) selection of the cultured cells from step (d) for the absence of the homologous sequence and/or the presence of the heterologous sequence, in particular the selectable marker,

(f) production of chimeric non-human mammals from the cells from step (e) by injection of these cells in mammalian blastocysts (preferably mouse blastocysts), transfer of the blastocysts in pseudo-pregnant female mammals (preferably mouse) and analyses of the resulting offspring for a modification of the NINTROX gene.

The mechanism of the homologous recombination (cf. R.M. Torres, R. Kühn, Laboratory Protocols for Conditional Gene Targeting, Oxford University Press, 1997) is used in step (c) to transfect embryonal stem cells. The homologous recombination between the DNA sequences present in a chromosome and new, added cloned DNA sequences enables the insertion of a cloned gene in the genome of a living cell in place of the original gene. By this method it is possible to obtain via chimeras animals which are homozygous for the desired gene or the desired gene portion of the desired mutation when embryonal germ cells are used.

The expression "embryonal stem cells" comprises any embryonal stem cells of a non-human mammal which are suitable for the mutation of the NINTROX gene. The embryonal mouse stem cells, in particular cells E14/1 or 129/SV, are preferred.

The term "vector" comprises any vector which by recombination with the DNA of embryonal stem cells enables a modification of the NINTROX gene. The vector preferably has a marker with which it is possible to select for present stem cells in which the desired recombination was made. Such a marker is e.g. the loxP/tkneo cassette which by means of the Cre/loxP system can be removed from the genome again.

In addition, the person skilled in the art knows conditions and materials to carry out steps (a) to (f).

A non-human mammal is provided by the present invention whose NINTROX gene is modified. This modification can be an elimination of the gene expression-regulatory function. By means of such a mammal or cells therefrom the gene expression-controlling function of NINTROX can be investigated selectively. Furthermore, it is possible to find substances, drugs and therapy approaches by which a selective influence can be exerted on the controlling function of NINTROX. Therefore, the present invention furnishes a basis for influencing the most varying diseases. Such diseases are e.g. limitations of the CNS functions which reach as far as mental retardation or the induction of cancer resulting from mistakes made in the control of cell proliferation. Furthermore, it should be possible to investigate in more detail and characterize the part of the hippocampus.

The following clones were deposited with DSMZ, *Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH* [German-type collection of micro-organisms and cell cultures], Mascheroder Weg 1b, D-38124 Braunschweig, on May 4, 1998:

DSM 12153:	*E. coli* JFC-484, partial sequence of the human NINTROX-cDNA
DSM 12154:	*E. coli* JFC-622, partial sequence of the murine NINTROX-cDNA
DSM 12155:	*E. coli* JFC-8D3, sequence of the human genomic NINTROX-DNA
DSM 12156:	*E. coli* JFC-P1-165, sequence of the murine genomic NINTROX-DNA

The figures show:

Figure 1: human sequence of the NINTROX gene

Figure 2: murine sequence of the NINTROX gene

Figure 3: sequence comparison between human (top) and murine (bottom) sequences
Solid bars: sequence-conserved regions (b)

Figure 4: energy diagram of the sequences from figure 3

Figure 5: homology comparison of NINTROX from various species
Figure 5a: partial sequence from hamster
Figure 5b: partial sequence from kangaroo
Figure 5c: partial sequence from macaca
Figure 5d: partial sequence from orangutan
Figure 5e: partial sequence from rat

Figure 5f: partial sequence from chimpanzee

The following example explains the invention:

Example 1: Identification and Characterization of the NINTROX Gene

For the identification of transcribed sequences from the region Xq2-7.3 to Yqter, whole RNA was initially isolated from various pig tissues (kidney, heart, spleen, liver, brain, etc.) and transcribed by means of oligo-dT into first strand cDNA. These complex cDNA samples which represent all of the genes transcribed in the respective tissue were then labeled radioactively and hybridized with the Xq27.3-Xqter-specific cosmid library. The cosmid library was in this connection analyzed in the form of cosmid clones arranged systematically on nylon membranes. Then, the cosmid DNA was isolated by the cosmid clones which had positive hybridization signals with the complex cDNA samples, was digested using EcoRI, separated by gel electrophoresis and transferred to nylon membranes. The restriction fragments which then had a positive hybridization with the complex, radioactively labeled cDNA samples were subsequently isolated and labeled radioactively and used for screening a fetal human cDNA library. By this, positive cDNA clones could be isolated which represented the transcript of the NINTROX gene.